



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of)

Michael A. Innis *et al.*)

Serial No. 09/639,273)

Filed: August 15, 2000)

Examiner: Eliane Lazar-Wesley

Group Art Unit: 1646

Atty. Dkt. No. 012441.00002

TECH CENTER 1600/2900

For: **Production of Tissue Factor Pathway Inhibitor**

DECLARATION UNDER RULE 132

I, Abba A. Creasey, hereby declare:

1. I am named as a joint inventor of the application referenced above.
2. I am an employee of Chiron Corporation, and all of the work described in the application was performed at Chiron Corporation.
3. In my experience, the activity of TFPI expressed in and purified from *Saccharomyces cerevisiae* as described in the above-identified application is similar to the activity of TFPI expressed in and purified from mammalian baby hamster kidney (BHK) cells.
4. Experiments conducted in our laboratories have demonstrated that the activity of TFPI expressed in and purified from *S. cerevisiae* as described in the application is also similar to the activity of TFPI expressed in and purified from *E. coli*.
5. The activity of TFPI expressed in and purified from *E. coli* is nearly identical to the activity of TFPI expressed in and purified from mammalian C127 cells. See Ho *et al.* (*Blood*, 95, 2000, 1973-1978; Exhibit 1), which teaches that TFPI expressed in and purified from mammalian C127 cells inhibits factor Xa activity similarly to TFPI expressed in and purified from *E. coli*. (See Fig. 2 and page 1975, column 2, lines 1-5.)
6. The TFPI expressed in and purified from *E. coli* used by Ho *et al.* was also used to determine that the *S. cerevisiae*-expressed and purified TFPI described in the application has activity that is similar to *E. coli*-expressed and purified TFPI. Ho *et al.* obtained the *E. coli*-

purified TFPI from Monsanto Company (Exhibit 1, page 1973, second column, lines 8-9, of the Materials and Methods.) Monsanto Company and Chiron corporation have a joint venture on the TFPI project, whereby Chiron manufactures *E.coli* expressed TFPI and provides purified TFPI to Monsanto and collaborators.

7. The activity of TFPI purified from C127 cells is similar to the activity of TFPI purified from BHK cells. See Wun *et al.* (*Thrombosis and Haemostasis*, 68, 1992, 54-59; Exhibit 2), which teaches that TFPI expressed in and purified from C127 cells has similar factor Xa inhibitory activity as TFPI expressed in and purified from BHK cells. (Page 56, column 2, lines 34-39.) See also Nordfang *et al.* (*Biochemistry*, 30, 1991, 10371-10376; Exhibit 3), which teaches that TFPI expressed in and purified from C127 cells has similar anticoagulant activity in a PT coagulation time assay to TFPI expressed in and purified from BHK cells. (Page 10371, column 2, lines 12-20.) Valentin *et al.* (*Blood Coagulation and Fibrinolysis*, 2, 1991, 629-635; Exhibit 4) also teaches that TFPI expressed in and purified from C127 cells has similar anticoagulant activity in a clotting time assay to TFPI expressed in and purified from BHK cells.

8. The activities of TFPI expressed in and purified from *S. cerevisiae* as described in the application, TFPI expressed in and purified from *E. coli*, TFPI expressed in and purified from C127 cells, and TFPI expressed in and purified from BHK cells are all similar. Therefore, the activity of the TFPI that is expressed in and purified from *S. cerevisiae* as described in the application is similar to the activity of TFPI expressed in and purified from BHK cells.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States; and further that false statements made willfully may jeopardize the validity of any patent issuing on an application in which the false statements were made.

Date 7/25/02

Abla A. Creasey